

AD

Award Number: W81XWH-04-1-0627

TITLE: The Role of HER-2 in Breast Cancer Bone Metastasis

PRINCIPAL INVESTIGATOR: Dihua Yu, MD, Ph.D.

CONTRACTING ORGANIZATION: University of Texas
M. D. Anderson Cancer Center
Houston, TX 77030

REPORT DATE: July 2005

TYPE OF REPORT: Final

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for Public Release;
Distribution Unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

20060302 026

REPORT DOCUMENTATION PAGE

*Form Approved
OMB No. 0704-0188*

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (DD-MM-YYYY) 01-07-05			2. REPORT TYPE Final		3. DATES COVERED (From - To) 07/01/04-06/30/05	
4. TITLE AND SUBTITLE The Role of HER-2 in Breast Cancer Bone Metastasis			5a. CONTRACT NUMBER			
			5b. GRANT NUMBER W81XWH-04-1-0627			
6. AUTHOR(S) Dihua Yu, MD, Ph.D. E-Mail: dyu@mdanderson.org			5c. PROGRAM ELEMENT NUMBER			
			5d. PROJECT NUMBER			
			5e. TASK NUMBER			
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) University of Texas M. D. Anderson Cancer Center Houston, TX 77030			8. PERFORMING ORGANIZATION REPORT NUMBER			
			10. SPONSOR/MONITOR'S ACRONYM(S)			
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			11. SPONSOR/MONITOR'S REPORT NUMBER(S)			
			12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited			
13. SUPPLEMENTARY NOTES						
<p>14. ABSTRACT: The major goal of thus Concept award is to define the role for the growth factor receptor, HER2, in breast cancer growth in the bone, thus involvement in bone metastasis. To achieve this, we proposed three tasks: Analysis of orthotropic model of breast cancer growth in the bone Immunohistochemical analysis of bone resorption and bone resorption markers Identification of potential HER2 targets involved in bone resorption We have found that over-expression of HER2 could enhance breast cancer bone metastases. In our pilot study, we found that when injected into the tibia of nude mice, breast cancer cells that over-express Her2 induced osteolytic lesions that were more aggressive than that of the parental cell line expressing low levels of HER2. Thus, HER2 may contribute to an increase in osteolytic activity of breast cancer bone metastases and further experiments may show that HER2 may serve as a therapeutic target for controlling breast cancer bone metastases. During the one-year funding period of this grant we experienced setbacks in our model system that hindered that completion of the tasks outlined in this award. We have remedied these obstacles and are actively pursuing the proposed experiments. Therefore, we would like to ask for a one-year non-funded extension of this grant to facilitate the completion of the outlined tasks.</p>						
15. SUBJECT TERMS						
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON USAMRMC	
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			19b. TELEPHONE NUMBER (include area code) 301-619-7325	

Table of Contents

Cover.....	1
SF 298.....	2
Introduction.....	4
Body.....	5
Key Research Accomplishments.....	7
Reportable Outcomes.....	7
Conclusions.....	7
References.....	8
Appendices.....	9

Introduction

It has long been recognized that breast cancers have the ability to invade and grow as metastases in the bone (Mundy GR 1997). In patients, the development of bone metastases causes extreme morbidity. HER2 (a.k.a. **ErbB2**) is a 185 kDa transmembrane glycoprotein, which is a receptor tyrosine kinase that belongs to the epidermal growth factor receptor subfamily (Yamamoto et al 1986). Overexpression of HER2 initiates aberrant activation, causing deregulation of downstream target genes. HER2 overexpression is found in approximately ~30% of breast cancers (Slamon et al 1987), and many other cancer types. Overexpression of HER2 correlates with poor clinical outcome and increased metastatic potential (Tan et al 1997) along with cancer recurrence. It has also been shown that bone marrow micrometastases express more HER2 than their primary tumor (Putz et al 1999) suggesting a selection bias for HER2 overexpressing cells in the bone microenvironment. Here we sought to understand the role of HER2 in breast cancer bone metastasis, thus seeking clinical impact through prognostic tests and preventative therapy, potentially decreasing HER2 mediated bone destruction.

To determine the role of HER2 in breast cancer bone metastasis, we proposed three Tasks. First, we sought to understand the biology of the bone environment when occupied by HER2 low and high expressing breast cancer cells, using intra-tibia injection. Next, in Task 2 we wished to perform immunohistochemical analysis, observing bone resorption in a site-specific manner for microscopic early stage bone resorption. Finally, we sought to identify potential HER2 targets causing bone resorption by using an unbiased approach to compare the expression levels of certain key targets

between the HER2 transfectants and their parental cell lines. Identification of novel targets would allow elucidation of the mechanism behind HER2-mediated bone resorption.

Body

In Task 1, we proposed to study HER2 over-expression involvement in breast cancer bone resorption/metastasis. To accomplish this task we proposed to inject 3 different HER2 low expressing parental breast cancer cells (MDA-MB-435, MCF-7, and MDA-MB-231) and their isogenic HER2 high expressing stable breast cancer cell lines (435.eB, MCF-7.eB, and 231.eB) into the tibias of female nude mice, monitoring growth and osteolytic activity through radiograph analysis. Unfortunately, after very limited passage number, western blot analysis revealed that

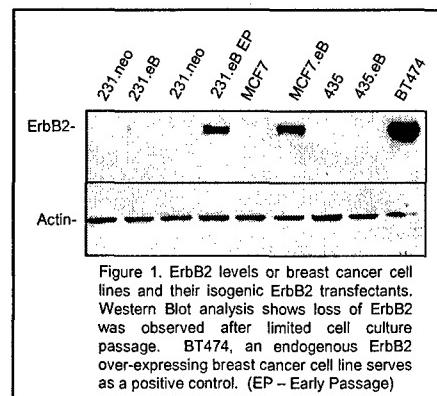


Figure 1. ErbB2 levels in breast cancer cell lines and their isogenic ErbB2 transfectants. Western Blot analysis shows loss of ErbB2 was observed after limited cell culture passage. BT474, an endogenous ErbB2 over-expressing breast cancer cell line serves as a positive control. (EP - Early Passage)

our 435.eB and 231.eB HER2 stable transfectants expressed only moderate to low levels of HER2 (Figure 1). To address this problem we created an HER2-expressing retrovirus (pLPCX-CMV-HER2) and infected the parental cell lines MDA-MB-435 to generate new 435.eB stable clones (Figure 2, clones 46, 71, and 115). To remedy the lack of expression of HER2 in the 231.eB cell line, we have collaborated with Dr. Patricia Steeg at NIH-NCI and obtained parental 231.EGFP and its HER2 transfectant, 231.ErbB2 1.1.

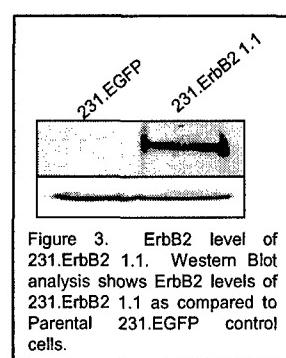
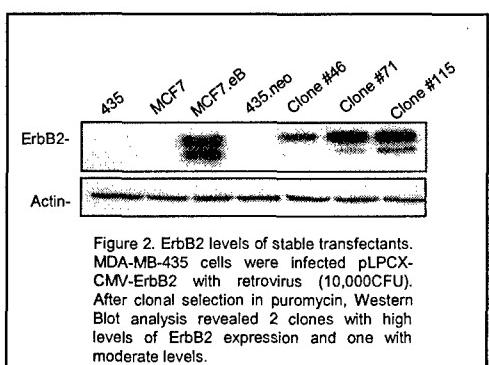


Figure 3. ErbB2 level of 231.ErbB2 1.1. Western Blot analysis shows ErbB2 levels of 231.ErbB2 1.1 as compared to Parental 231.EGFP control cells.

(Figure 3). In addition, these two cell lines express green fluorescence protein (GFP), which will also allow for in vivo imaging of occult metastasis and growth within the bone for future experimentation. Both cell lines have been characterized and are ready for experimentation. At the time of the original submission of this proposal, we proposed to use the breast cancer cell line MCF-7 and its stable HER2 transfectant MCF-7.eB. We have since learned that the MCF-7 breast cancer cell line is not a useful model for monitoring breast cancer cell growth within the bone. MCF-7 cells are ER positive and the mice require the supplementation of estrogen for proper MCF-7 cell growth. The effects of estrogen on bone growth, turnover and development is well established and add additional factors that create an unsuitable and complicated scenario for our studies. After the generation of our new 435.eB transfectants, we conducted a pilot in vivo study. As proposed, we first investigated and monitored tumor growth in the microenvironment of the bone, comparing, HER2 high expressing breast cancer cells, 435.eB to their parental MDA-MB-435, HER2 low expressing counterparts. We injected, each breast cancer cell line (5×10^5 cells /injection) intratibially into NCRNU-M female nude mice (Taconic Farms Inc., Germantown, NY), 5 mice per cell line, 10 mice total. After an initial growth period of one week, we took X-rays of each mouse once a week using Faxitron Specimen Radiography System (Figure 4, Appendix Figure 1,2).

Radiographic analysis indicated that both MDA-MB-435 and 435.eB were capable of growing in the bone microenvironment however the 435.eB breast cancer cells had an

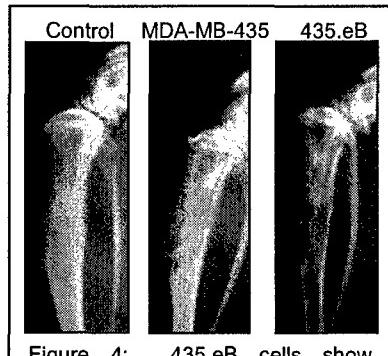


Figure 4: 435.eB cells show increased osteolysis *in vivo*. MDA-MB-435 and 435.eB (5×10^5) cells were injected intratibially into female nude mice. Weekly x-rays were taken for 8 weeks, figure depicts final image at 8 weeks.

increase in bone destruction and osteolytic activity. In addition, both cell lines produced distant metastases observed during necropsy, including lung and kidneys, suggesting that HER2 over-expressing 435.eB has an advantage in growing in the unique environment of the bone when compared to MDA-MB-435. Data collected from this pilot experiment is quite exciting and with our properly established system, we are currently continuing the original tasks outlined in the proposal. Therefore, we would like to **ask for a one-year non-funded extension of this grant** to facilitate the completion of this proposal.

Key Research Accomplishments

- New stable cell lines overexpressing HER2, 435.eB and 231.eB were created facilitating a proper model system for our proposed tasks
- Injection of MDA-MB-435 and 435.eB cells into the tibias of nude mice produced osteolytic lesions. Animals injected with 435.eB cells had an increase in osteolysis and bone degradation.

Reportable Outcomes

None

Conclusions

After the development and characterization of our new model system, we now have breast cancer cell lines with appropriate levels of HER2 to carry out the proposed tasks. In the pilot study, we were able to demonstrate that both MDA-MB-435 and 435.eB grow in the microenvironment of the bone. In addition we saw an increase of bone degradation from the 435.eB cells suggesting that over-expression of HER2 in breast

cancer cells causes an increase of osteolytic activity. Further experimentation and completion of the proposed tasks will determine the role of HER2 in breast cancer bone metastasis.

References

Mundy GR 1997 mechanisms of bone metastasis. *Cancer* 80:1546-1556

Yamamoto T, Ikawa S, Akiyama T, Semba K, Nomura N, Miyajima N, Saito T, Toyoshima K. Similarity of protein encoded by the human c-erb-B-2 gene to epidermal growth factor receptor. *Nature*. 1986 Jan 16-22; 319(6050): 230-4.

Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. *Science*. 1987 Jan 9; 235(4785): 177-82.

Tan M, Yao J, Yu D. Overexpression of the c-erbB-2 gene enhanced intrinsic metastasis potential in human breast cancer cells without increasing their transformation abilities. *Cancer Res*. 1997 Mar 15; 57(6): 1199-205.

Putz E, Witter K, Offner S, Stosiek P, Zippelius A, Johnson J, Zahn R, Riethmuller G, Pantel K. Phenotypic characteristics of cell lines derived from disseminated cancer cells in bone marrow of patients with solid epithelial tumors: establishment of working models for human micrometastases *Cancer Res*. 1999 Jan 1; 59(1): 241-8.

Appendix

Figure 1: MDA-MB-435 breast cancer cell line (5×10^5 cells /injection) were injected intratibially into 5 NCRNU-M female nude mice. After an initial growth period of one week, X-rays of each mouse were taken once a week using Faxitron Specimen Radiography System.

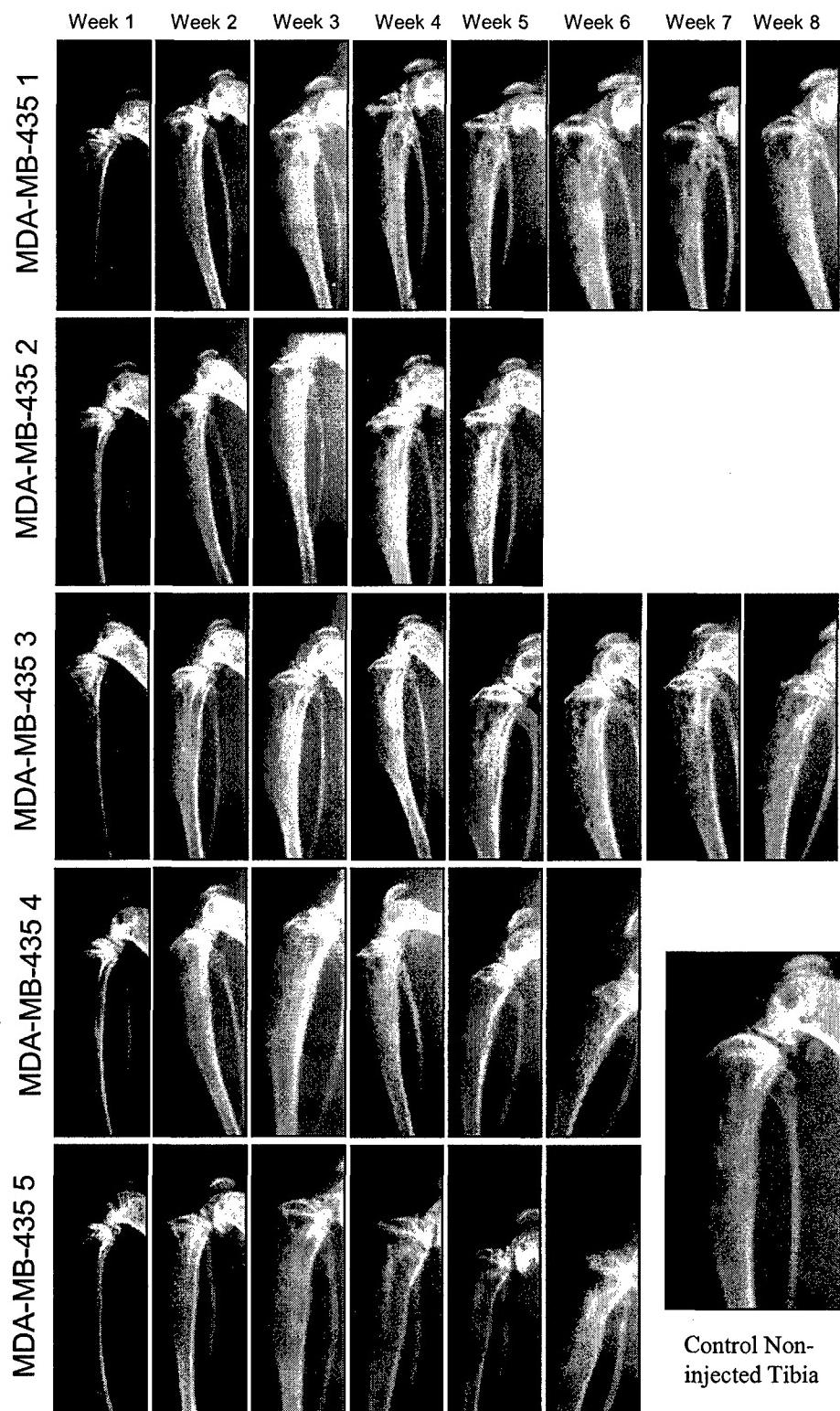


Figure 2: 435.eB breast cancer cell line (5×10^5 cells /injection) were injected intratibially into 5 NCRNU-M female nude mice. After an initial growth period of one week, X-rays of each mouse were taken once a week using Faxitron Specimen Radiography System.

